

Prevalence and Intensity of *Gyrodactylus arcuatus* Bychowsky, 1933 (Monogenea) Infestations on the Three-Spined Stickleback, *Gasterosteus aculeatus* L., 1758

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Received: 04.04.2002

Abstract: *Gyrodactylus arcuatus* Bychowsky, 1933 was identified on the three-spined stickleback, *Gasterosteus aculeatus* L., 1758 in the present study, which was carried out between January and May 2000. A total of 151 fish specimens were examined. The infestation prevalence and mean intensity levels were 80.2% and 35.44 ± 8.87 parasites per fish, respectively. The infestation prevalence and mean intensity levels were highest in February and May, respectively. No statistically significant preference for either sex of fish was determined. The mean intensity of *G. arcuatus* was highest on the gills, followed by the fins and skin. Significant differences were observed in the distribution of *G. arcuatus* over the body parts studied with respect to the length classes of fish.

Key Words: *Gyrodactylus arcuatus*, *Gasterosteus aculeatus*, three-spined stickleback, Sırakırkağaçlar stream

***Gyrodactylus arcuatus* Bychowsky, 1933 (Monogenea) Enfestasyonunun Dikence Balığı, *Gasterosteus aculeatus* L., 1758'deki Oranı ve Yoğunluğu**

Özet: Ocak ve Mayıs 2000 tarihleri arasında yürütülen bu çalışmada, dikence balıkları (*Gasterosteus aculeatus* L., 1758) üzerinde *Gyrodactylus arcuatus* Bychowsky, 1933 türünün varlığı tespit edildi. Toplam olarak 151 adet balık incelendi. Ortalama enfestasyon oranı ve yoğunluğu, sırasıyla % 80,2 ve $35,44 \pm 8,87$ parazit / enfekte balık olarak belirlendi. Enfestasyon oranı (%) ve intensitesi sırasıyla Şubat ve Mayıs aylarında en yüksek değerlerinde kaydedildi. İstatistiki olarak, balığın cinsiyetine göre bir enfestasyon tercihi belirlenmedi. *G. arcuatus*'un enfestasyon intensitesi en fazla solungaçlar, ardından da yüzgeçler ve vücut yüzeyinde kaydedildi. Boy sınıfları dikkate alındığında, *G. arcuatus*'un balığın vücut kısımlarındaki yayılışlarında önemli farklılıklar belirlendi.

Anahtar Sözcükler: *Gyrodactylus arcuatus*, *Gasterosteus aculeatus*, dikence balığı, Sırakırkağaçlar deresi

Introduction

Monogeneans are widespread throughout freshwater and marine habitats. Members of the genus *Gyrodactylus* parasitize a great variety of fish species inhabiting both environments. *Gyrodactylus* are viviparous and infect a wide range of teleost and amphibian hosts and live ectoparasitically on the skin, fins and gills of fish. In general, gyrodactylus are present as single species infections of hosts and it is suggested that *Gyrodactylus* species show strict specificity to their hosts (1-3). There are very rare reports, however, on the coexistence of several gyrodactylus species on a single host, but in different parts of the host bodies (3). Harris (4) listed 13

species of gyrodactylus from the three-spined stickleback throughout the world, including *Gyrodactylus arcuatus* Bychowsky, 1933.

The three-spined stickleback, *Gasterosteus aculeatus* L., 1758, inhabits fresh, brackish and salt waters throughout marine coasts between about 35°N and 70°N in Europe. Its diet includes copepods, crustaceans and oligochaetes (5). Sticklebacks breed in spring and temperature may be one of the influential factors in their growth (6). Gyrodactylids are considered among the serious pathogens of fish and mortalities in sticklebacks in experimental infections were also reported by Lester and Adams (7).

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In this study, a comprehensive investigation was conducted on the occurrence of *Gyrodactylus arcuatus* on the three-spined stickleback found in a small stream flowing into the Black Sea at Sinop in Turkey.

Materials and Methods

Fish were collected by gill net and/or cast net from Sırakırkağaçlar stream. The stream is euryhaline, especially during winter and early spring when the stream mouth joins with the Black Sea due to a rise in the water level. The fish were transported live in a 25-l insulated container to Ondokuz Mayıs University, Sinop Fisheries Faculty Parasitology Laboratory for ectoparasitic examination. A total of 151 fish were investigated (1 of which was immature). The sticklebacks were weighed, the total length measured and allocated to length classes and their sex determined post-mortem. Skin, gills and fins were examined with a stereomicroscope and the parasites were counted for each body part of the host fish.

Gyrodactylus specimens were relaxed in tap water, fixed in 10% formaldehyde and transferred to 5% glycerol in 70% ethanol after 1 week. In addition, at least 10 specimens of *Gyrodactylus* were placed on glass microscope slides with a drop of 1/4000 formalin and the cover slip placed over the parasite. Excess solution was removed using a filter paper. The whole mount was examined using a phase contrast microscope under oil immersion at x100 magnification. Some specimens were also fixed under the cover slip by adding a drop of ammonium picrate-glycerin to the edge of the cover slip (8). Identification of the parasite was performed according to the characteristics described by Malmberg (2) and Harris (4).

The terms infestation prevalence (%) and mean intensity are used in accordance with the definitions established in Bush et al. (9). The prevalence (%) was calculated as the percentage of the total number of fish infested out of the total number of fish examined. The mean intensity was calculated as the average number of parasites in the total number of infected fish.

A Kruskal-Wallis test (nonparametric ANOVA) was performed to find significant differences in the mean intensity values of *G. arcuatus* for infestation sites, length classes of fish and the months in which this study was conducted. The difference between parasite loading on

male and female fish was tested by the Mann-Whitney U test (10). All statistical analyses were performed at the significance level of 5% using the statistical program GraphPad InStat 3.00.

Despite the several attempts made during the months following May, no fish were collected due to their possible movement upwards into deeper waters.

Results

Overall infestation prevalence (%) and mean intensity levels of *G. arcuatus*

Throughout the investigation period, *G. arcuatus* was found to be the only gyrodactylus species infesting *Gasterosteus aculeatus* (Figure 1). Of the 151 fish specimens examined, 121 were infested with a prevalence of 80.2% and mean intensity level of 35.44 ± 8.87 *G. arcuatus* per infested fish (Table 1). The distributions of *G. arcuatus* over the body parts of the host fish were also recorded. The differences between the infestation intensities recorded in each part of the fish body were statistically significant ($P < 0.05$) (Table 1).

On the other hand, infestation prevalence fluctuated slightly during the examination period, but stayed above 62.5%, which was recorded in January (Figure 2). Mean intensity levels gradually increased, in contrast to the prevalence levels, throughout the study period up to May, when the maximum level of 165.8 ± 127.4 *G. arcuatus* per infested fish was recorded.

Statistically significant differences were found in the intensity levels between the months January-April, February-March, February-April and March-April ($P < 0.05$).

The distribution of *G. arcuatus* with respect to the length classes of the host fish

Within the 5 different fish length classes studied (Table 1), infestation prevalence was lowest (62.5%) in the 55-59 mm length class while it was above 73% in the rest. The maximum mean intensity level was 52.33 ± 20.49 *G. arcuatus* per infested fish in the 65-69 mm length class (Table 1).

The infestation prevalence and the mean intensity values determined from the body parts of the host fish are presented in Table 2. Despite the statistically significant distribution of *G. arcuatus* with respect to all

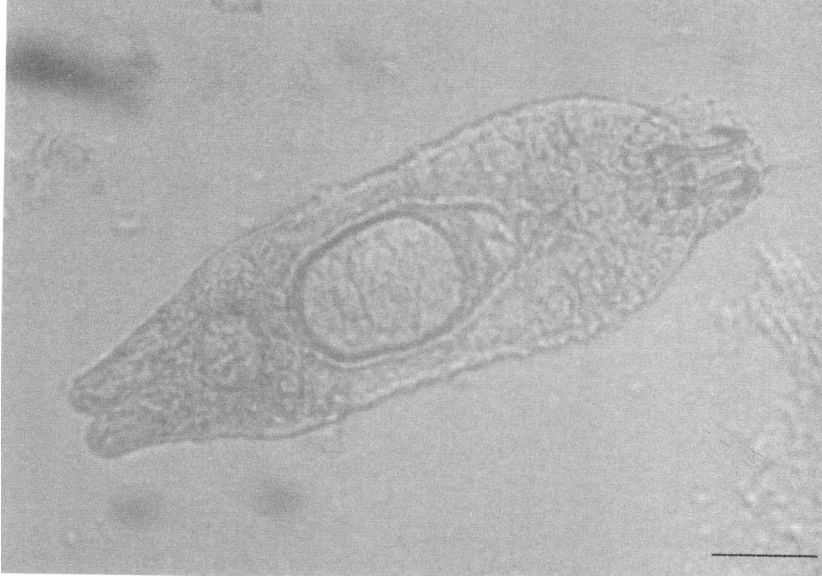


Figure 1. An individual of *Gyrodactylus arcuatus* Bychowsky, 1933. Scale bar: 100 μ m.

Table 1. Infestation prevalence (%) and mean intensity levels of *Gyrodactylus arcuatus* determined from the three-spined stickleback (*Gasterosteus aculeatus*) caught in Sırakırkaçlar stream in Sinop, Turkey.

	Infestation prevalence (%)	Mean intensity \pm S.E.
Infestation site		
Gills (n = 82)	54.3	24.4 \pm 12.6*
Fins (n = 95)	62.9	19.3 \pm 2.3*
Skin (n = 101)	66.9	4.6 \pm 0.5*
Sex of fish ¹		
Female (n = 116)	81.9	37.5 \pm 11.1
Male (n = 34)	73.5	29.2 \pm 7.4
Length classes of fish (mm)		
\leq 59 (n = 21)	66.7	14.1 \pm 4.0
60 – 64 (n = 29)	89.6	23.4 \pm 3.2
65 – 69 (n = 61)	85.2	52.3 \pm 20.5
70 – 74 (n = 30)	73.3	33.1 \pm 8.5
75 \geq (n = 10)	80.0	10.8 \pm 3.1
Overall (n = 151)	80.2	35.4 \pm 8.9

Mean values with an asterisk in a column represent statistically significant differences ($P < 0.05$)

¹ means that 1 fish was immature out of 151 fish examined

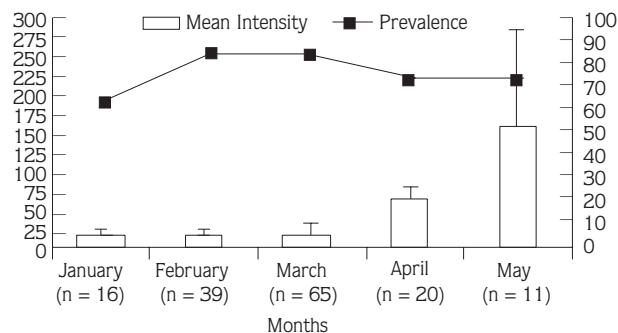


Figure 2. Monthly infestation prevalence (%) and mean intensity levels of *Gyrodactylus arcuatus* determined from *Gasterosteus aculeatus* caught in Sırakırkağaçlar stream in Sinop, Turkey.

length classes of the host fish ($P < 0.05$), no significant differences between the mean intensity values of each length class were determined ($P > 0.05$). On the other hand, the differences between the intensity levels determined from each body part with respect to the length classes were statistically significant ($P < 0.05$) (Table 2).

The distribution of *G. arcuatus* on host fish of different sexes

The infestation prevalence and mean intensity levels were both higher in females than in males (Table 1). However, the difference between the mean values was not statistically significant ($P > 0.05$). The distributions of *G. arcuatus* over the body parts of the host fish are presented in Table 2. Fins were the site least favoured by

G. arcuatus on both sexes (Table 2). No statistically significant preference for any studied body part of either sex was determined ($P > 0.05$).

Discussion

Throughout the investigation period in the present study, *G. arcuatus* was the only gyrodactylus species found infesting *Gasterosteus aculeatus*, with a prevalence of 80.2% and mean intensity level of 35.44 ± 8.87 *G. arcuatus* per infested fish. High infestation rates of *Gyrodactylus* species studied from natural populations have also been reported by several authors (11-15). Rokicki and Vojtkova (16) reported an 80% infestation prevalence for *G. arcuatus* on the three-spined stickleback in Poland. Sultana (17) also determined an overall infestation prevalence of 90.83% and mean intensity of 18.92 for *G. gasterostei* Glaser, 1974 infestations on the same host species. Thus, the prevalence and mean intensity levels recorded in the present study agree with those reported by the above-mentioned authors.

Generally, gyrodactylids reproduce continuously throughout the year. However, studies on the seasonal occurrence of *Gyrodactylus* species show that there is a strict influence of temperature on the prevalence and intensity of infestations which generates a definite seasonal cycle of the parasite. While some species show a maximum prevalence in spring (18,19), in summer (14,15) or in winter (4), Sultana (17) determined 100%

Table 2. Infestation prevalence (%) and mean intensity levels of *Gyrodactylus arcuatus* on the body parts of the three-spined stickleback (*Gasterosteus aculeatus*) caught in Sırakırkağaçlar stream in Sinop, Turkey, in different fish length classes and sexes.

	No. of fish examined	No. of fish infested			Prevalence (%)			Mean intensity levels \pm S.E.		
		Gills	Fins	Skin	Gills	Fins	Skin	Gills	Fins	Skin
Fish length (mm)										
≤ 59	21	11	9	11	52.4	42.9	52.4	10.5 ± 4.3	$6.2 \pm 2.2^*$	$1.9 \pm 0.4^*$
60-64	29	18	23	23	62.1	79.3	79.3	9.4 ± 1.4	15.2 ± 2.3	3.3 ± 0.5
65-69	61	36	43	41	59.0	70.5	67.2	41.7 ± 28.6	$22.1 \pm 3.4^*$	5.3 ± 0.9
70-74	30	16	19	19	53.3	63.3	63.3	13.0 ± 5.3	27.9 ± 8.3	3.8 ± 0.8
$75 \leq$	10	1	1	6	10.0	20.0	60.0	6.00	3.00	$12.3 \pm 3.9^*$
Fish sex										
Female	116	74	82	63	73.3	70.7	54.3	27.6 ± 16.3	19.8 ± 2.6	4.4 ± 0.3
Male	34	20	18	18	58.8	52.9	52.9	14.6 ± 6.5	18.3 ± 5.0	5.6 ± 1.6

Mean values with an asterisk in a column represent statistically significant differences ($P < 0.05$)

of prevalence in April for *G. gasterostei* and the level was above 90% in most months except June-August. Harris (4) assumed that this decline was due to the disappearance or death of adult fish in that period. The schooling behaviour of hosts before the breeding season in winter and spring is an important factor in parasite population increases. Direct contact is one of the main routes for *Gyrodactylus* infestations (20) and could be another factor in higher prevalence and intensity levels. A number of authors have suggested that the prevalence and intensity of gyrodactylid monogeneans are greatly influenced by the life cycle stage of the host fish (2-4,21,22). A gradual increase in intensity levels up to May in the present study could be a result of the above-mentioned factors and increased temperatures in spring (4 °C in January and 24.8 °C in May). In addition, some discharges from the scarce settlements around the stream may be another factor in the increase, especially during late spring and summer, when the water level dropped considerably and the connection with the Black Sea was broken. At this point, a solution to decrease parasite intensity may be the re-establishment of the connection to ensure water current between the stream and the Black Sea. It was not possible for us to collect fish throughout a 1-year period due to the possible movement of three-spined sticklebacks upstream and into deeper waters. Thus, the pattern of infestation is not known at these times.

There are some contradictions concerning the host and site specificity of gyrodactylids: while some authors consider gyrodactylids to be narrowly specific (1,2), others consider them less host specific (20). Several fish species, such as *Neogobius melanostomus* Pallas, 1811, *Mugil cephalus* L., 1758 and *Liza aurata* Risso, 1810, were also

present at the sampling site where this study was conducted but none was found to be infected by this parasite (unpublished data). The mean intensity value of *G. arcuatus* was highest on the gills, followed by the fins and skin in the present study. Several authors also recorded this broad site specificity for gyrodactylids (23-25).

The infestation prevalence remained above 62.5% and intensity levels showed a slight fluctuation as the size of the fish increased. Similar results were obtained by Sultana (17) for the infestation of *G. gasterostei* on the three-spined stickleback. Our findings are also in agreement with the results of Chappell (21) and Harris (4).

The sex of fish has been shown to affect their parasitic infections, because of differing colour, hormonal states and mucus between female and male fish (26). Those authors determined higher prevalences on females than males or on immature fish. This was also the case in the present study. There is no other study showing any preference for either host sex.

The data presented here contribute to the knowledge on *Gyrodactylus arcuatus* Bychowsky, 1933 and add some valuable information on its occurrence on both sexes, the length class of fish and the body parts of the host *Gasterosteus aculeatus*. However, its occurrence in freshwater, euryhaline or marine environments, its full-scale seasonal variations and its reproductive biology remain to be elucidated.

Acknowledgement

This study was financed by Ondokuz Mayıs University under project number S-076.

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